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Antiepileptic Drug Development: II. Anticonvulsant Drug Screening

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INTRODUCTION

Despite optimal use of the 16 antiepileptic drugs marketed in the United States, many patients with epilepsy fail to experience seizure control and others do so only at the expense of significant toxic side effects. In the early 1970s, no convincing evidence had been published that the 13 primary antiepileptic drugs marketed in the U.S. at that time controlled the seizures of more than 50% or improved more than 75% of the patients with epilepsy (Coatsworth, 1971). The availability of carbamazepine and clonazepam in 1974 and 1975, respectively, brought improved seizure control to many patients, and evidence suggests that the recently marketed valproic acid will not only control the seizures of additional patients but will also reduce the side effects of therapy. Notwithstanding the beneficial effects of the current drugs, there is still a need for new antiepileptic drugs with more selective anticonvulsant effects and less toxicity.

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A survey of pharmaceutical firms conducted by the Epilepsy Branch of the National Institute of Neurological and Communicative Disorders and Stroke in the late 1960s uncovered few new drugs in preclinical development for the treatment of epilepsy. A number of pharmaceutical firms had discontinued screening new chemical compounds for anticonvulsant activity, and others had failed to develop compounds that were known to be active anticonvulsants. The major cause of this lack of interest in antiepileptic drug development was its high cost relative to the size of the available market. A complicating factor was the uncertainty of predicting the clinical efficacy of new anticonvulsant compounds.

Another survey indicated that academic medicinal chemists interested in synthesizing anticonvulsant compounds had no ready source for anticonvulsant screening. Consequently, the interest of synthetic chemists had been diverted to other areas of pharmacology.

After reviewing the surveys' findings, the U.S. Surgeon General's Public Health Service Advisory Committee on the Epilepsies (1967) recommended that the Epilepsy Branch undertake efforts to increase the involvement of both industry and academia in the discovery and development of

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new anticonvulsant drugs (Krall et al., 1978). Thus, in 1975, the Epilepsy Branch established the Anticonvulsant Screening Project and began screening new chemical compounds for anticonvulsant activity and potential development as antiepileptics. The Project's goal is to identify more potent and less toxic anticonvulsant compounds whose structures are novel and whose mechanisms of action differ from those of the currently marketed drugs.

Both pharmaceutical firms and medicinal chemists from academic institutions are invited to submit chemicals for testing. By providing a testing service for the academic medicinal chemist, the Project hopes to spur interest in developing new chemistry for anticonvulsant drug synthesis. For the pharmaceutical firms, the Project offers to evaluate anticonvulsant compounds against a much greater number and variety of anticonvulsants than any one firm can gather and thereby hopes to place the best anticonvulsant compounds in a more favorable atmosphere for development into drugs. By performing its testing in a standardized, consistent manner, the Screening Project aims to develop a uniform, computer-stored, and retrievable data bank for study of the structure–activity relationships of anticonvulsants and the predictability of anticonvulsant tests.

Between January 1, 1975, and December 31, 1976, the Anticonvulsant Screening Project accessioned 1,495 compounds. The results of the evaluation of these compounds, as of May 1, 1978, and the design of the testing program are described here.

PROCEDURES

Compound Solicitation and Submission

Solicitation of compounds from the pharmaceutical industry and academic medicinal chemists has been accomplished by large scale mailings, published announcements, and personal contacts by staff and consultants of the Epilepsy Branch. All testing is done without charge.

Participating pharmaceutical firms and the National Institute of Neurological and Communicative Disorders and Stroke sign an agreement that describes the testing to be performed and grants the NINCDS, in exchange for said testing, rights limited to the development of data supporting antiepileptic utility. Nonprofit organizations need not sign an agreement. The submittor retains the patent rights to all compounds offered for testing, in accordance with the legal precedent of Applegate and Howell vs. Scherer, Frensch, and Stahler, Court of Customs and Patent Appeals, 141 USPQ 796, June 11, 1964.

A 500 mg sample of each compound is requested for the initial evaluation. When necessary, adequate evaluation can be accomplished with smaller samples. The compound's structure, molecular weight, and molecular formula, as well as data on its purity and solubility, are required. Compounds are submitted to the Epilepsy Branch, where they are cataloged, weighed, and divided into two samples. One sample remains in the Branch to protect against loss or breakage. The other is shipped to the University of Utah College of Pharmacy (E.A.S.) for testing.

Testing Methods

All compounds are solubilized or suspended in either 0.9% sodium chloride solution or 30% aqueous polyethylene glycol 400. Compounds are administered intraperitoneally in a volume of 0.01 ml/g body weight to adult male CF #1 mice (obtained from the Charles River Breeding Laboratories, Wilmington, Massachusetts 01887) weighing an average of 20 g. Two tests for anticonvulsant activity and one test for minimal neurological deficit are performed.

Maximal Electroshock Seizure Test (MES)

Maximal seizures are elicited by a 60 Hz alternating current of 50 mA (five to seven times that necessary to elicit minimal seizures) delivered for 0.2 sec via corneal elec-

trodes. A drop of 0.9% sodium chloride instilled in each eye prior to application of the electrodes assures adequate electrical contact; it also reduces the incidence of fatalities to near zero. Maximal seizures are produced in all normal mice. The maximal seizure typically consists of a short period of initial tonic flexion and a prolonged period of tonic extension (especially of the hind limbs) followed by terminal clonus. The typical seizure lasts approximately 22 sec. Failure to extend the hind limbs to an angle with the trunk greater than 90° is defined as protection (Swinyard et al., 1952).

Subcutaneous Pentylenetetrazol (Metrazol®) Seizure Threshold Test (scMet)

This test produces threshold or minimal (clonic) seizures. Pentylenetetrazol, as a 0.85% solution in 0.9% sodium chloride, is administered subcutaneously in a loose fold of skin on the back of the neck in a dose of 85 mg/kg. The animal is observed for 30 min after pentylenetetrazol administration, during which time seizures are produced in approximately 97% of normal mice. Absence of a single 5 sec episode of clonic spasms (a threshold seizure) is defined as protection (Swinyard, 1972).

Rotorod Toxicity Test

This test is designed to detect minimal neurological deficit (Dunham and Miya, 1957). The mouse is placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm. Normal mice can remain indefinitely on a rod rotating at this speed. Neurological deficit (e.g., ataxia, sedation, hyperexcitability) is indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three trials.

Testing Protocol

The testing protocol consists of three

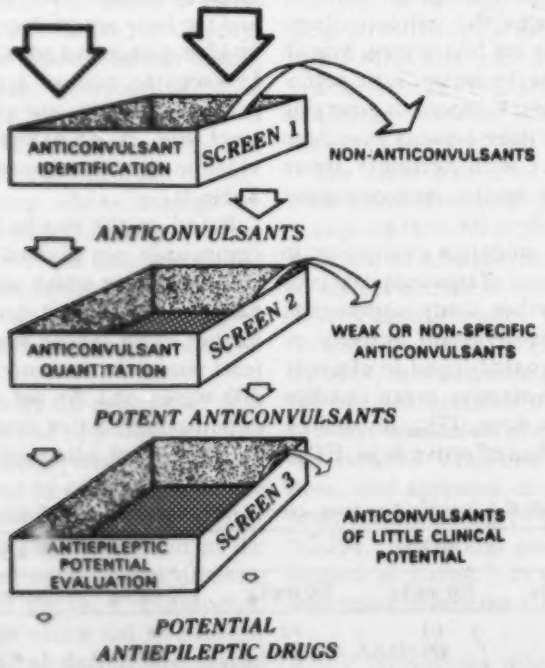


FIG. 1. Schematic depicting how potential antiepileptic drugs are separated from new chemical entities.

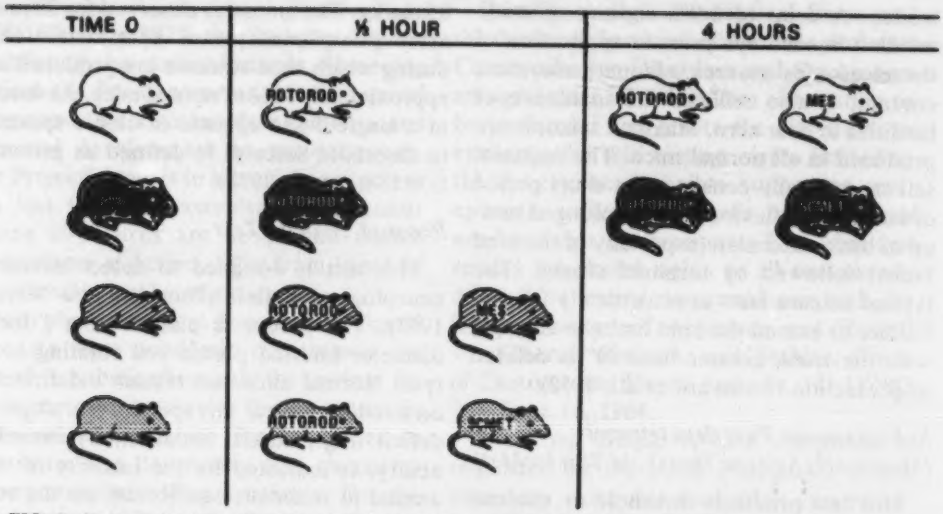


FIG. 2. Screen: At time 0 the test drug is administered to 4 mice (tests are performed at 30, 100, and 300 mg/kg). The maximal electroshock seizure test (MES), the subcutaneous (Metrazol®) pentylenetetrazol test (scMet), and the Rotorod toxicity test (Rotorod) are performed 30 min and 4 hr later.

successive screens (Fig. 1). Screen 1 is designed to identify anticonvulsant activity. Screen 2 quantitates the anticonvulsant activity identified in the first screen. Screen 3 is used to evaluate the antiepileptic potential of the most potent compounds emerging from screen 2. The three screens have been designed to select with certainty those compounds whose median anticonvulsant dose is much less than 100 mg/kg.

All compounds undergo evaluation in screen 1. The purpose of this appraisal is to eliminate from further study compounds that have no anticonvulsant activity or those that exhibit toxicity, and to estimate the potency and protective index (median minimal neurotoxic dose, TD_{50}, divided by MES or scMet median effective dose, ED_{50}) of those that are anticonvulsants. Screen 1 employs doses of 30, 100, and 300 mg/kg, and the tests are performed at 30 min and 4 hr after compound administration. In order to keep the cost of screen 1 evaluations low, only 12 mice are used, 4 at each dose level (Fig. 2). An example of the data provided in the initial assessment is shown in Table 1.

Based on the results of the first screen, compounds are divided into three groups (Fig. 3). Those which are inactive or cause neurological deficit are eliminated from further study. Those which are active in at least one of the anticonvulsant tests at 30 or 100 mg/kg and do not cause neurological deficit at their active dose proceed to screen 2. Those not eliminated or directed to

TABLE 1. *Sample data from screen 1 (anticonvulsant identification)*

Test	30 Min			4 Hr		
	30 mg/kg	100 mg/kg	300 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
MES	1/1	1/1	1/1	0/1	1/1	1/1
scMet	0/1	0/1	0/1	0/1	0/1	0/1
Rotorod	0/4	1/4	4/4	0/2	0/2	1/2

Values represent number of mice protected or toxic divided by number of mice tested.

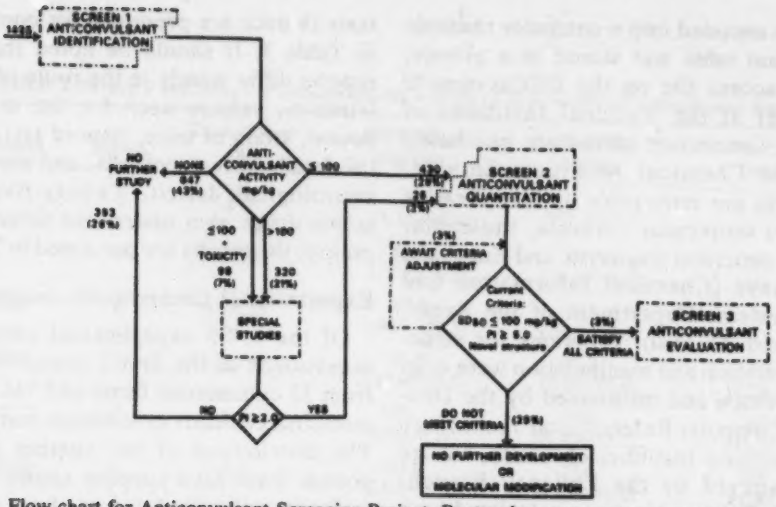


FIG. 3. Flow chart for Anticonvulsant Screening Project. Rectangles represent screens and special studies. Diamonds represent decision criteria. ED_{50}: Median effective dose; PI: Protective index (Rotorod TD_{50}/anticonvulsant ED_{50}). The number and percentage of the 1,495 compounds reaching specific points in the project are shown on the flow lines.

screen 2 are compared with tested congeners. If a compound represents a novel structure or appears to be free of toxicity, it undergoes special studies (further testing at appropriate dosage intervals) to better define its protective index. Those with an estimated protective index of at least 2.0 proceed to screen 2. Further study of compounds in this group whose analogs are more active, or whose estimated protective index is low, is carried out only when additional data will contribute to structure–activity studies or when new data suggest that these compounds might have a therapeutic advantage.

Screen 2 quantitates the estimate of anticonvulsant activity and neurotoxicity. The MES and scMet ED_{50}s and the Rotorod TD_{50} are determined in this screen. In the case of a compound with activity in only one anticonvulsant test, evaluation in the other is carried out up to and sometimes beyond doses that produce neurological deficit. Compounds which fail to produce minimal neurological deficit are tested whenever possible at doses up to 10 times their lowest anticonvulsant ED_{50}. Determination of the MES ED_{50} and the scMet ED_{50} is carried out at the time of peak effect in the MES test, except when preliminary testing indicates that scMet activity occurs at another time. The Rotorod TD_{50} is determined at the time of peak neurological deficit.

The most potent compounds emerging from screen 2 become candidates for screen 3 evaluation, which further assesses their potential as antiepileptic drugs. The goal is to collect sufficient data to justify further developmental studies. Currently, screen 3 includes evaluation of anticonvulsant activity after oral administration, evaluation of the species specificity of anticonvulsant activity, delineation of drug effects between the Rotorod TD_{50} and the median lethal dose, and appraisal of anticonvulsant and toxic effects during 7–14 days of administration. Additional studies may be performed in screen 3 as experience dictates their value in defining antiepileptic potential.

Data Analysis

The structure of each accessioned com-

pound is encoded into a computer readable connection table and stored in a private, limited access file on the DECsystem-10 computer at the National Institutes of Health. Connection tables are purchased from the Chemical Abstracts Service. Structures are retrievable by identification number, molecular formula, molecular weight, structural fragments and rings, and CIDS keys (Chemical Information and Data System, Department of the Army, Edgewood Arsenal). Programs for structure generation and manipulation were originally written and maintained by the Division of Computer Research and Technology at the National Institutes of Health, but are now managed by the Epilepsy Branch. Median effective doses are calculated by a FORTRAN probit analysis program written by the staff of the Epilepsy Branch.

The results of testing are stored in limited access (secured) files on the DECsystem-10 computer. The data are retrievable by identification number, supplier, category of results, and actual values. FORTRAN programs for the data storage and retrieval were written by one of the authors (R.L.K.). The structure and data retrieval programs are on-line and interactive, so that searches immediately produce both the specific structure and the data characteristics of each compound.

RESULTS

Standardization of Testing Methods

Seventy known compounds were selected for testing in order to serve as comparisons for the experimental compounds. These standards consisted of the primary antiepileptic drugs marketed in the United States, other drugs known to be anticonvulsants, structural analogs of known anticonvulsants, and drugs with central nervous system activity quite different from anticonvulsants. The results of a screen 2 evaluation of the 16 U.S. marketed antiepileptic drugs and one adjunct are shown in Table 2. Published reports on similar tests in mice are presented for comparison in Table 3. It should be noted that these reports differ widely in the route of administration, vehicle used for the test compound, strain of mice, time of test, technical details of test methods, and measure of neurological deficit. Twenty-five CNS-active drugs also underwent screen 2 appraisal; the results are presented in Table 4.

Experimental Compounds—Accessions

Of the 1,495 experimental compounds accessioned in the first 2 years, 749 came from 32 commercial firms and 746 from 36 medicinal chemists in academic institutions. The distribution of the number of compounds from each supplier shows that the majority submitted fewer than 11 compounds and only six submitted more than 75 (Fig. 4). Not unexpectedly, academic institutions outnumbered industry in the submission of 10 or fewer compounds.

Experimental Compounds—Results

Screen 1 evaluation of the 1,495 experimental compounds has been completed (see Fig. 3). Four hundred thirty compounds (29%) had anticonvulsant activity at 30 or 100 mg/kg without signs of neurotoxicity and have undergone or await screen 2 evaluation. Three hundred twenty compounds (21%) demonstrated anticonvulsant activity at 300 mg/kg, and 98 (7%) had more potent anticonvulsant action accompanied by neurotoxicity. Special studies showed that 25 of these 418 compounds had estimated protective indices sufficient to warrant scheduling for screen 2. Thus, 455 (31%) of the compounds evaluated in the first screen were advanced to the second stage of appraisal. Six hundred forty-seven compounds (43%) showed no anticonvulsant activity in screen 1 and will not be studied further.

Screen 2 evaluations have been completed in 352 of the 430 compounds with anticonvulsant activity at 30 or 100 mg/kg. Two hundred eighty-two compounds had

TABLE 2. *Screen 2 evaluation of anticonvulsant activity of U.S. marketed antiepileptic drugs*

Compound	Solvent	Time of test[a] (hr)	MES ED_{50} (mg/kg)	scMet ED_{50} (mg/kg)	Rot TD_{50} (mg/kg)
Phenytoin	S	2.0	9.50 (8.13–10.4)	Not active	65.5 (52.5–72.1)
Ethotoin	P	0.25	85.5 (72.0–92.2)	48.0[b] (42.2–63.0)	171 (144–250)
Mephenytoin	P	0.5/0.25	60.5 (49.5–70.3)	30.5 (19.7–39.5)	154 (133–179)
Phenobarbital	S	1.0/0.5	21.8 (15.0–25.5)	13.2 (5.87–15.9)	69.0 (62.8–72.9)
Metharbital	P	0.5	129 (108–151)	10.0 (6.47–15.2)	67.3 (43.7–130)
Mephobarbital	P	4.0	33.8 (21.1–42.3)	23.6 (19.1–31.2)	195 (174–209)
Primidone	P	3.0/24.0[c]	11.4 (4.16–16.7)	58.6[b] (36.0–83.6)	680 (647–717)
Trimethadione	P	1.0/0.5	627 (538–705)	300 (265–325)	819 (652–1,096)
Paramethadione	P	0.5	237 (218–249)	63.0 (48.2–75.0)	291 (266–313)
Ethosuximide	S	0.5	>1,000	130 (111–150)	441 (383–485)
Methsuximide	P	0.5	76.3 (62.6–89.3)	68.3[b] (61.5–82.5)	188 (160–236)
Phensuximide	P	0.5	112 (104–131)	50.3 (20.7–64.5)	232 (187–267)
Phenacemide	P	1.0/0.5	87.3 (73.9–99.5)	116 (70.8–150)	421 (337–549)
Diazepam[d]	P	0.5/0.25	19.1 (12.9–28.8)	0.165 (0.128–0.206)	7.30 (4.60–8.70)
Clonazepam	P	0.5	92.7 (44.9–189)	0.009 (0.005–0.017)	0.18 (0.16–0.23)
Carbamazepine	P	0.25	8.81 (5.45–14.1)	Not active	71.6 (45.9–135)
Valproic acid	S	0.25	272 (247–338)	149 (123–177)	426 (369–450)

[a] Time of MES and scMet tests/Rotorod test; single entry indicates all tests performed at same time.
[b] Values are substantially greater than those cited in earlier reports because originally tested samples were contaminated by benzodiazepines.
[c] Toxicity increases with time.
[d] Adjunct only.
MES ED_{50}, Maximal electroshock seizure test, median effective dose.
scMet ED_{50}, Subcutaneous Metrazol® seizure threshold test, median effective dose.
Rot TD_{50}, Rotorod toxicity test, median neurotoxic dose.
S, 0.9% Sodium chloride.
P, 30% Polyethylene glycol 400.
Numbers in parentheses show 95% confidence interval.

TABLE 3. *Published reports of anticonvulsant activity of U.S. marketed antiepileptic drugs*

Compound	MES ED_{50} (mg/kg)		scMet ED_{50} (mg/kg)		TD_{50} (mg/kg)	
Phenytoin	9.1	Goodman et al., 1953*a*	NA	Goodman et al., 1953*a*	90	Swinyard et al., 1952
	10	Swinyard et al., 1952	NA	Swinyard et al., 1952	110	Wolf et al., 1962
	12.8	Raines et al., 1973				
	16	Wolf et al., 1962				
Ethotoin	350	Everett, 1972	350	Everett, 1972	400	Everett, 1972
Mephenytoin	33	Swinyard et al., 1952	40	Wolf et al., 1962	95	Swinyard et al., 1952
	37	Goodman et al., 1953*a*	55	Swinyard et al., 1952	200	Wolf et al., 1962
	46	Wolf et al., 1962				
Phenobarbital	14	Dick and Mitchell, 1967	9.8	Vida et al., 1973*a*	51.0	Goodman et al., 1953*b*
	20	Vida et al., 1973*a*	12.5	Raines et al., 1973	55	Swinyard et al., 1952
	28	Goodman et al., 1953*b*	15	Dick and Mitchell, 1967		
	50	Raines et al., 1973	15.5	Goodman et al., 1953*b*		
Metharbital	30	Vida et al., 1973*b*	18	Vida et al., 1973*b*	>200[a]	Vida et al., 1973*b*
Mephobarbital	16	Vida et al., 1973*b*	24	Vida et al., 1973*b*	64	Swinyard et al., 1952
	20.8	Goodman et al., 1953[a]	25	Swinyard et al., 1952		
	22	Swinyard et al., 1952				
Primidone	13.0	Goodman et al., 1953*b*	74.0	Goodman et al., 1953*b*	1,120	Goodman et al., 1953*b*
Trimethadione	~620	Swinyard et al., 1952	~480	Swinyard et al., 1952	~640	Swinyard et al., 1952
	630	Goodman et al., 1953*a*				
Paramethadione	~160	Swinyard et al., 1952	~120	Swinyard et al., 1952	~145	Swinyard et al., 1952

TABLE 3.—(*Continued*)

Ethosuximide	>800	Banziger and Hane, 1967	20	Chen and Bass, 1964	400	Chen et al., 1963
	2,070	Chen et al., 1963	135	Chen et al., 1963		
			500	Banziger and Hane, 1967		
Methsuximide	84	Chen et al., 1963	~60	Chen et al., 1963	~105	Chen et al., 1963
Phensuximide	115	Clemson et al., 1970	~80	Chen et al., 1963	~120	Chen et al., 1963
	183	Chen et al., 1963	134	Clemson et al., 1970		
Phenacemide	~110	Swinyard et al., 1952	~150	Swinyard et al., 1952	~620	Swinyard et al., 1952
	109	Goodman et al., 1953*a*				
Diazepam[b]	6.4	Banziger, 1965	0.27	Swinyard and Castellion, 1966	25	Randall et al., 1961
	12	Randall et al., 1961	1.37	Banziger, 1965	57	Swinyard and Castellion, 1966
	18.7	Swinyard and Castellion, 1966	2	Randall et al., 1961		
Clonazepam	400	Banziger, 1965	0.036	Swinyard and Castellion, 1966	285	Swinyard and Castellion, 1966
	>2,400	Swinyard and Castellion, 1966	0.16	Banziger, 1965		
Carbamazepine	7.5	Julien and Hollister, 1975	11.5	Davis et al., 1964	66	Davis et al., 1964
	11.7	Davis et al., 1964	16	Julien and Hollister, 1975	176	Coyne and Cusic, 1968
	16	Theobald et al., 1968	20	Theobald et al., 1968		
	20	Coyne and Cusic, 1968	NA	Coyne and Cusic, 1968		
Valproic acid	235	Shuto and Nishigaki, 1970	120	Shuto and Nishigaki, 1970	290	Shuto and Nishigaki, 1970
	260	Swinyard, 1964	120	Swinyard, 1964	415	Swinyard, 1964

[a] Median hypnotic dose.
[b] Adjunct only.
MES ED_{50}, Maximal electroshock seizure test, median effective dose.
scMet ED_{50}, Subcutaneous Metrazol® seizure threshold test, median effective dose.
TD_{50}, Median neurotoxic dose.
NA, Not active.

TABLE 4. *Screen 2 evaluation of CNS-active compounds*

Compound	Solvent	Time of test[a] (hr)	MES ED_{50} (mg/kg)	scMet ED_{50} (mg/kg)	Rot TD_{50} (mg/kg)
Acetazolamide	S	2.0	46.6 (34.9–54.2)	69.0 (0.870–230)[b]	>1,000[c]
Albutoin	P	1.0/0.5	99.9 (62.7–115)	16.7 (12.2–22.4)	56.7 (51.2–118)
Aminoglutethimide	P	0.5	15.3 (12.8–19.4)	39.3 (28.1–72.8)	89.9 (78.3–97.2)
Amitriptyline HCl	S	0.5	9.60 (8.21–13.0)	Not active[d]	21.6 (20.2–23.8)
Benzchlorpropamide	P	1.0/0.5	185 (158–380)	177 (127–255)	278 (245–6,062)[e]
Chloral hydrate	S	0.5	199 (179–213)	89.0 (64.8–119)	181 (159–196)
Chlorazepate dipotassium	P	0.5	41.4 (29.6–49.1)	0.324 (0.232–0.502)	42.8 (36.0–46.1)
Chlordiazepoxide	S	1.0/0.25	29.2 (25.4–36.8)	1.61 (1.21–2.63)	39.5 (24.4–70.0)
Chlorpromazine	S	1.0	63.9 (9.24–88.9)	4.38 (2.84–5.96)	7.90 (3.43–11.7)
Dimethoxymethylphenobarbital	P	4.0/8.0	38.6 (34.7–43.9)	33.1 (—)[f]	386 (332–463)
Flurazepam	S	0.5	47.1 (43.5–56.6)	0.473 (0.287–0.623)	17.8 (5.68–36.0)
Imipramine HCl	S	0.5	28.0 (22.4–53.3)	Not active	83.2 (73.1–146)
Lidocaine HCl	S	0.5	26.6 (24.8–28.1)	Not active	110 (97.7–129)
Magnesium sulfate	S	0.5	934 (884–986)	>1,000	708 (635–749)
Meprobamate	P	0.5	120 (107–131)	31.5 (22.8–38.6)	125 (112–135)

TABLE 4—(*Continued*)

Methaqualone	P	0.5	52.2 (45.5–59.4)	33.3 (27.9–38.8)	53.8 (45.4–60.2)
Mexiletine HCl	S	0.25	9.90 (7.93–11.7)	Not active	41.6 (35.7–53.0)
Nitrazepam	P	1.0/0.5	18.1 (8.62–35.8)	0.087 (0.069–0.134)	1.47 (1.16–1.70)
Oxazepam	P	0.5	81.3 (72.8–94.2)	0.360 (0.280–0.450)	13.3 (10.6–15.3)
Pentobarbital	S	0.25	32.8 (18.8–37.8)	13.0 (10.6–15.2)	25.8 (23.4–30.5)
Pheneturide	P	0.5/2.0	77.4 (60.7–147)	41.5 (32.8–53.5)	355 (290–504)
Phethenylate sodium	P	0.5	31.2 (27.6–49.2)	Not active	143 (121–180)
Procyclidine HCl	P	0.5	33.6 (30.6–35.8)	Not active	36.1 (29.1–42.0)
Sulthiame	P	1.0	38.3 (36.3–40.1)	338 (162–505)	>1,500[e]
Trihexyphenidyl HCl	P	0.5	75.7 (58.9–82.5)	Not active	98.2 (88.2–111)

[a] Time of MES and scMet tests/Rotorod test; single entry indicates all tests performed at same time.
[b] Slope 4.5 and standard error of slope 2.3 account for large confidence interval.
[c] Tested at 30 min and 1, 2, and 4 hr.
[d] Inconsistent protection from 15 to 50 mg/kg.
[e] Slope 11.5 and standard error of slope 5.7 account for large confidence interval.
[f] Slope 2.5 and standard error of slope 1.6; confidence interval not definable from data.
MES ED_{50}, Maximal electroshock seizure test, median effective dose.
scMet ED_{50}, Subcutaneous Metrazol® seizure threshold test, median effective dose.
Rot TD_{50}, Rotorod toxicity test, median neurotoxic dose.
P, 30% Polyethylene glycol 400.
S, 0.9% Sodium chloride.
Numbers in parentheses show 95% confidence interval.

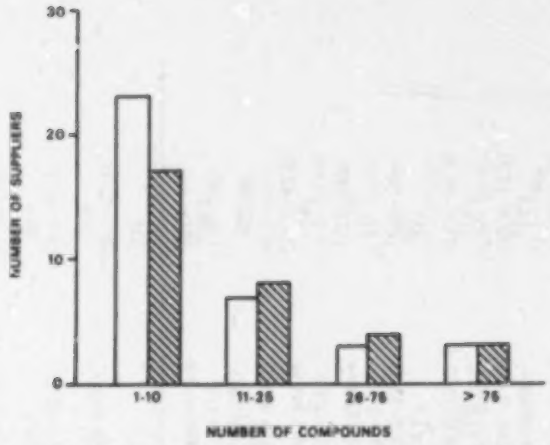


FIG. 4. Distribution of number of compounds from academic (□) and industrial (■) suppliers.

an ED_{50} of less than 100 mg/kg in either one or the other of the anticonvulsant tests. Seventy-one compounds had an MES ED_{50} of less than 100 mg/kg, but the scMet ED_{50} exceeded 100 mg/kg. Thirty compounds had an scMet ED_{50} of less than 100 mg/kg but an MES ED_{50} of more than 100 mg/kg. Both the MES ED_{50} and the scMet ED_{50} were less than 100 mg/kg in 181 compounds.

Sixteen of the experimental compounds whose screen 2 testing is complete have anticonvulsant activity at 75 mg/kg or less in at least one test and a protective index greater than 5.0. Thirteen of these are under study in screen 3, and the remaining three are candidates for future study.

Quality Control

Three aspects of Project performance have been monitored: variability of screen 1 results, rate of false negative screen 1 results (active compounds which were missed), and correspondence between screen 1 and screen 2 results.

Five randomly selected compounds were subjected to repeated, blind screen 1 evaluations (25 evaluations, approximately 2% of the initial appraisals) to determine the variability in screen 1 test results. The pooled results of these multiple evaluations are presented in Table 5. Because anticonvulsant activity at either the 30 or 100 mg/kg dose is sufficient to advance a compound

TABLE 5. *Comparison of initial and 25 repeated screen 1 evaluations*

Repeated results	All Tests			MES			scMet			Rotorod		
>300	0	0	33	0	0	12	0	0	14	0	0	7
300	1	21	1	0	9	0	0	4	1	1	8	0
100												
30	19	0	0	4	0	0	6	0	0	9	0	0
Dose (mg/kg)	30 100	300	>300	30 100	300	>300	30 100	300	>300	30 100	300	>300
	Initial Results											

Each repeated evaluation is recorded in the column of the lowest active dose in the initial trial and the row of the lowest active dose in the repeated trial.

to screen 2 study, the results at these dose levels have equal impact on the further testing of any compound. Consequently, the results at 30 and 100 mg/kg were summed.

Only two of the 25 repeated screen 1 trials differed from the initial screen 1 results. In one case, the repeated trial showed Rotorod toxicity at 300 mg/kg instead of 100 mg/kg as observed in the initial trial. In the other case, one repeated scMet test showed activity at 300 mg/kg, whereas the initial trial failed to demonstrate activity at any of the doses tested. Neither of these cases revealed a change in activity greater than one dose level of testing. None of the compounds whose initial testing showed either no effect or activity at only high doses proved to have activity at doses of 100 mg/kg or less on repeated trials.

To estimate the number of false negative screen 1 test results, 33 randomly selected compounds (approximately 2% of all compounds tested) which showed no activity in screen 1 were blindly reevaluated. A false negative result was defined as failure of a compound to demonstrate activity in screen 1 despite having anticonvulsant activity at doses of 100 mg/kg or less. Of the 33 compounds inactive on the first screen 1 evaluation, two demonstrated scMet activity and one both scMet and MES activity at 300 mg/kg in the repeated trial. None of the compounds showed activity at 30 or 100 mg/kg. In this sample, then, there was no evidence of any significant occurrence of false negative results.

To monitor the correspondence between screen 1 and screen 2 results, the first 226 compounds that had completed screen 2 appraisal were classified into three groups on the basis of activity found in screen 1. In the first, "potent," group were those compounds showing activity at 30 or 100 mg/kg. The second, "moderately potent," group included compounds with activity at 300 mg/kg only. The third, "impotent," group showed no activity at any dose level. The MES, scMet, and Rotorod results of each screen 1 test were so classified. Any screen 2 test in which a compound failed to demonstrate activity up to the highest doses tested was assigned an arbitrary ED_{50} of 500 mg/kg.

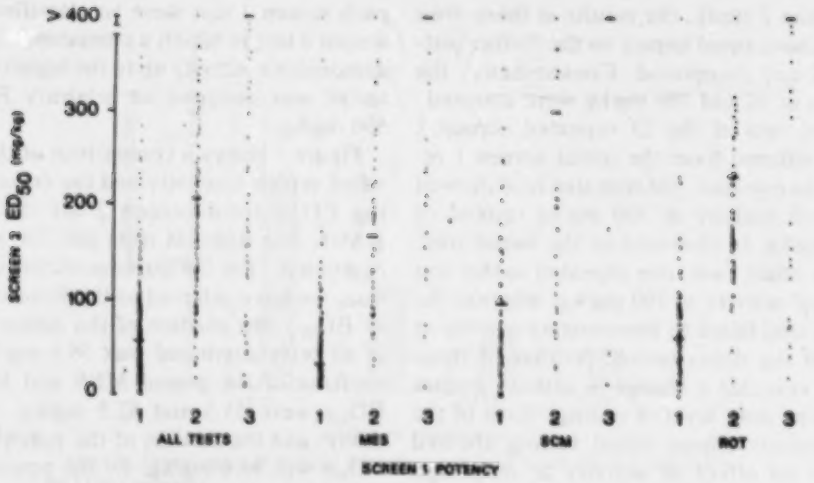
Figure 5 shows a comparison of the classified screen 1 activity and the corresponding ED_{50}s from screen 2 for the MES, scMet, and Rotorod tests and for all tests combined. (For the purpose of this illustration, we have referred to the Rotorod TD_{50} as ED_{50}.) The median of the potent ED_{50}s of all tests combined was 56.4 mg/kg; the medians of the potent MES and Rotorod ED_{50}s were 33.3 and 62.5 mg/kg, respectively, and the median of the potent scMet ED_{50}s was 94.6 mg/kg. Of the potent MES and Rotorod ED_{50}s, 9.9% and 15.9%, respectively, exceeded 100 mg/kg, compared to 49.2% of the potent scMet ED_{50}s.

Eleven ED_{50}s were less than 100 mg/kg for nine compounds that were impotent in screen 1. A false negative result in screen 1 was revealed by one Rotorod TD_{50}; animals which should have failed the first Rotorod test did not. The 300 mg/kg dose of all the other compounds and the 100 mg/kg dose of four compounds were lethal in screen 1, preventing observation of activity. The narrow margin between anticonvulsant and lethal effects compromised the potential of these compounds for development as antiepileptic drugs.

In the group with moderate potency in screen 1, only 18.9% of the scMet ED_{50}s were less than 100 mg/kg, compared to 27.9% of the MES ED_{50}s. Because two of the moderately potent compounds had MES ED_{50}s of less than 60 mg/kg, we reviewed all 150 compounds in this group and selected for screen 2 evaluation the 22 with MES activity. Four of these had ED_{50}s of less than 100 mg/kg and protective indices greater than 2.5.

DISCUSSION

The goal of any screening project for drug development is the discovery of more effec-



MEDIAN ED_{50} (mg/kg)	58.4	208.1	>400.	33.3	188.0	>400.	94.8	300.5	>400.	62.6	232.2	>400.
NUMBER OF ED_{50}S	268	154	186	142	43	41	114	37	75	88	74	64
PERCENT ED_{50}S ≤100	72.8	14.9	5.4	90.1	27.9	48	50.8	18.9	2.5	84.1	5.4	7.8
PERCENT ED_{50}S >100	27.4	85.1	94.6	9.9	72.1	95.1	49.2	81.1	97.5	15.9	94.6	92.2

FIG. 5. Comparison of results from first and second screens. Each point represents a screen 1 and screen 2 test result. Screen 1 activity is classified as 1 (potent), 2 (moderately potent), and 3 (impotent). The median ED_{50} of each group is indicated by a horizontal bar.

tive and less toxic compounds than those available. The ultimate success of a screening effort depends on the compounds it evaluates, the biological assays it utilizes, and the protocol which directs its testing.

The kinds of compounds tested in an anticonvulsant screening project can significantly bias the results. If the compounds are dominated by a few chemical series, then the results will reflect the activity of the lead compounds. If these lead compounds are active, then the project is likely to produce a large number of compounds with similar anticonvulsant and, consequently, clinical antiepileptic activity. On the other hand, if the structures are varied, then the likelihood that many will be active is lessened, but the chance of finding a new active structure is enhanced.

Only a small proportion of compounds tested in the Anticonvulsant Screening Project belong to the same structural classes as the currently marketed drugs (Table 6). The majority of tested compounds represent new directions in the synthesis of anticonvulsant agents, and 10 of the 13 compounds under advanced study for antiepileptic potential represent five structural classes that are different from those of known antiepileptic drugs. The large number of active anticonvulsant compounds from new structural classes was unexpected.

When multiple biological assays are

TABLE 6. *Number of analogs of antiepileptic drugs accessioned in the Anticonvulsant Screening Project, 1975–1976*

Hydantoins	39
Barbiturates	2
Oxazolidinediones	6
Succinimides	25
Acetylureas	27
Benzodiazepines	22
Dibenzazepines	24

available for measuring similar pharmacological activity, the choice of assays can bias the evaluation of a compound's activity. The selected assays should provide the best possible prediction of clinical efficacy and should at least be able to detect the activity of all clinically effective drugs. Interpretation of the results is easier if the assays measure pharmacological characteristics which correlate with clinical usefulness. The chosen assays should perform these functions efficiently, that is, with as little overlap among tests as possible.

The selection of assays to be used in the Anticonvulsant Screening Project was based on the following criteria: The tests had to measure a compound's ability to modify both maximal seizures and threshold (minimal) seizures, since these pharmacological effects are relevant to both clinical efficacy and mechanisms of drug action. The tests had to be reliable (reproducible). They had to identify all known clinically effective agents, as well as all other agents identified as anticonvulsants by other reliable tests. The tests had to be easily, quickly, and inexpensively performed. The testing laboratory had to have expertise in their performance and had to be familiar with the multitude of variables that affect the results.

The use of animal seizures to study compounds for anticonvulsant activity and antiepileptic potential dates back to 1882, when direct faradic stimulation of the canine cerebral cortex was used to study the effects of potassium bromide, atropine, and a cinchona alkaloid (Albertoni, 1882). Experimental techniques were not used for large scale screening of compounds until Merritt and Putnam conducted their studies in the late 1930s (Putnam and Merritt, 1937; Merritt and Putnam, 1938). The single test utilized in their program was an electroshock seizure model in cats.

The success of Merritt and Putnam's technique created interest in finding simpler, less costly animal models that would require less drug. A variety of rodent tests of electroshock seizure threshold were reported by 1945 (Goodman et al., 1945). The value of testing potential anticonvulsants in the presence of an abnormally lowered threshold was also explored (Goodman et al., 1945; Swinyard, 1949).

A number of phylogenetic and ontogenetic factors involved in seizure production and protection were identified. Comparisons between the mouse and rat response to antiepileptic drugs showed no qualitative differences (Swinyard et al., 1952), although quantitative and even qualitative differences are known to occur among different species of mice. (For a review of phylogenetic differences in seizure susceptibility, see Servit, 1972.) Striking changes in seizure susceptibility and response to drugs were found to occur during maturation. Thus, both the choice of animal and its stage of development were found to be important (Vernadakis and Woodbury, 1969; Purpura, 1972).

Pentylenetetrazol was introduced for anticonvulsant screening by Richards and Everett (1944) when they demonstrated trimethadione's unique ability to prevent seizures which it evoked. A variety of other convulsant chemicals (e.g., picrotoxin, thujone, methionine sulfoximine) were subsequently employed, but none was widely used or as effective as pentylenetetrazol. (For a review of systemic chemical convulsants, see Stone, 1972).

A further modification of the electroshock seizure threshold test was the low frequency or "psychomotor" electroshock test (Toman et al., 1952). The similarity of the evoked reaction to complex partial seizures and its inhibition by phenacemide, a drug thought to be particularly effective against this seizure type, initially supported its reputation as a selective screening method.

In 1953, Brown and colleagues investigated the difference between the psychomotor seizure test and the minimal electro-

shock threshold test, using eight clinically available drugs. They found that the two tests were qualitatively equivalent measures of seizure threshold and that comparative rankings of the drugs were remarkably similar. They were unable to find any correlation between the results of the psychomotor test and efficacy in psychomotor seizures and concluded that there was no foundation for its use as a specific test.

It soon became clear that no one of the available tests was capable of identifying all of the known clinically effective drugs; hence, batteries of tests were being used for screening purposes. Investigators began to construct profiles of drug action based on batteries of seizure models (Goodman et al., 1949; Swinyard, 1949). These batteries generally included a maximal electroshock test, a threshold pentylenetetrazol test, and at least one test of 60 Hz electroshock threshold or the low frequency electroshock seizure threshold test. The composition of these batteries reflected developing knowledge that modification of the pattern of maximal seizures and elevation of the seizure threshold represented two distinct physiological mechanisms. The MES test was recognized as a measure of the first effect and the scMet test as a measure of the second (Goodman et al., 1953*a*). Profiles of the clinically useful drugs correlated well with the kinds of seizures for which they were effective, but as the number of tested drugs increased it became apparent that there was overlapping in their clinical and experimental action (Toman et al., 1952).

Throughout the next decade, numerous investigators sought ways to improve the correlation between test results and clinical efficacy and to eliminate redundant tests. Chen and co-workers (1954) evaluated the supramaximal electroshock and psychomotor electroshock seizure tests and the intravenous pentylenetetrazol (PTZ) infusion (Orloff et al., 1949), supramaximal PTZ, and subcutaneous PTZ seizure tests, using 10 drugs. They concluded that the two supramaximal seizure tests provided similar information and that there was significant redundancy among the intravenous PTZ, subcutaneous PTZ, and psychomotor electroshock tests. The subcutaneous PTZ seizure model was preferred over the other seizure threshold tests for the practical reasons of time, ease of performance, reliability, and ease in expressing results. They expressed preference for the electroshock procedure for producing maximal seizures, primarily because it provided a much clearer contrast between the actions of phenytoin and other drugs.

Jenney and Pfeiffer (1956) performed a similar study of the MES test and the timed intravenous PTZ and strychnine infusion tests. They demonstrated that antipentylenetetrazol activity correlated well with clinical efficacy in childhood petit mal seizures, that anti-MES activity correlated with efficacy in grand mal and partial seizures, and that activity in all three tests indicated possible activity in psychomotor or adult petit mal seizures.

Over the succeeding 10 years, a number of authors reported their experience with batteries of screening tests (Sorel, 1957; Bastian, 1961; Spinks and Waring, 1962; Toman and Everett, 1964; Swinyard, 1969). Despite differences among the reports, all pointed out certain factors common to the screening of potential anticonvulsant drugs. First, it is often impossible to distinguish between sedative-hypnotics, tranquilizers, and anticonvulsants on the basis of their anticonvulsant test results alone. Then, too, all of these tests measure one or both of two apparently independent seizure endpoints. One is the threshold for evoking a seizure and the other is modification of seizure pattern. Also, methods for testing drugs for antiabsence activity seem less specific than those available for testing drugs for efficacy in major motor seizures. The primary antiabsence drugs are not potent inhibitors of maximal seizures.

The results of our evaluation of the antiepileptic drugs marketed in the U.S. correlate well with the published results, with only a few exceptions (Tables 2 and 3). In general, the major differences in Rotorod data concern test systems, mouse species, methods, and endpoints. Eight compounds had scMet ED_{50}s that are lower than those in the published reports, but the remaining scMet results are equivalent. There was no consistent difference in methods that could explain this disparity. The MES ED_{50}s vary only slightly from those in the published reports, and there is no pattern to the variation.

These data demonstrate that the antiepileptic drugs marketed in the U.S. are adequately identified by the MES and scMet tests. Clinically useful agents marketed elsewhere are also active in these tests (see Table 4). Because the primary drugs for absence seizures (ethosouximide, valproic acid, and trimethadione) are not the most potent inhibitors of scMet seizures and have even less inhibitory effect in MES seizures, our screening protocol is not likely to identify this characteristic profile of activity. This reflects our intention to find drugs with different mechanisms of antiepileptic action.

The reproducibility of the MES, scMet, and Rotorod test results is well documented by the quality control data and has been substantiated by regularly repeated studies of the standards. We have examined the reasons for the poor correspondence between the screen 1 and screen 2 results of the scMet test. The correlation between scMet ED_{50} and the slope of the scMet regression line is −0.820 (Krall, *in press*), much greater than the same correlation for the MES (−0.198) and Rotorod (−0.465) tests. This means that the slope becomes flatter as the scMet ED_{50} increases. Consequently, the dose range over which a compound shows scMet activity enlarges as the ED_{50} increases, and this results in a greater predictive error for the small sample of activity measured by screen 1. Examination of the data from the compounds in the potent scMet group whose ED_{50}s exceeded 100 mg/kg reveals that this type of error accounts for the major portion of the difference in correspondence between the screen 1 and screen 2 scMet tests and the MES and Rotorod tests.

An additional source of error is introduced by the method; a few normal mice (less than 3%) will fail to have a threshold convulsion after the standard dose of PTZ. This test result is falsely interpreted as a demonstration of anticonvulsant activity. Higher doses of PTZ result in maximal seizures in some animals and complicate the measurement of threshold modification.

Contamination of test agents by very potent substances can result in false positive results. We have found that as little as 3.25 μg of potent benzodiazepines carried over in glassware may result in protection against scMet seizures and that much greater amounts can be detected in glassware after routine cleaning procedures (Swinyard and Woodhead, *in press*). This problem has only affected the scMet test because these compounds exhibit potency in this test which exceeds that of the MES and Rotorod tests by an order of magnitude of 100–1,000. Since observing this problem, we have reviewed all of our data to eliminate this source of error.

We have investigated alternative methods of measuring threshold elevation, including the timed intravenous PTZ infusion test, and have not found them superior to the scMet test. We have accepted the poorer correspondence between screen 1 and screen 2 scMet results because the false positives that result are easily discovered in the quantitation testing of screen 2.

The protocols of screens 1 and 2 have proved to be fast and accurate procedures for the identification and quantitation of anticonvulsant activity. The cost of a screen 1 appraisal, including animal acquisition and care, personnel, and overhead, is ap-

proximately $40. The cost of a screen 2 evaluation averages $400. The projected cost of a screen 3 analysis is approximately $3,000. Our findings that screen 1 is appropriately eliminating compounds from further study and that screen 2 selectively identifies compounds for further study justify the three-screen protocol in a project whose goal is the discovery of potent (ED_{50} much less than 100mg/kg) agents.

The selection of the historically powerful MES and scMet anticonvulsant tests raises the possibility that the Screening Project will select drugs with mechanisms of action and clinical effects similar to those of the marketed drugs, all of which have activity in, and for the large part were selected by, one or the other of these tests. The use of these tests may compromise one goal of the Project, identification of drugs with new mechanisms of action. However, no new methods tied to proposed mechanisms of anticonvulsant action have been developed to the point that they can be utilized in large scale screening. We believe that these two anticonvulsant tests combined with the Rotorod neurotoxicity test provide the best screening battery available. We are continually investigating potential new screening tests in screen 3.

SUMMARY

By means of the maximal electroshock seizure (MES) test, the subcutaneous pentylenetetrazol (PTZ) seizure threshold test, and the Rotorod minimal neurotoxicity test, the Anticonvulsant Screening Project has evaluated the activity of 1,495 experimental compounds accessioned in the first 2 years. A three-screen protocol for appraising these compounds has proved reliable, fast, and inexpensive. Preliminary data show that 430 of these compounds have good anticonvulsant activity. Completed evaluations of 352 identified 16 that have anticonvulsant activity at doses less than 75 mg/kg and protective indices greater than 5.0.

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RÉSUMÉ

Le programme de dépistage des médicaments antiépileptiques (anticonvulsant screening project), basé sur les deux tests des crises provoquées chez l'animal par l'électrochoc et l'injection sous-cutanée de pentylenetetrazol et sur le test Rotorod de neurotoxicité minimale, a permis d'évaluer les propriétés antiépileptiques de 1,495 produits expérimentés pendant les deux premières années de son activité. Le protocole opératoire à trois niveaux ainsi utilisé s'est avéré sûr, rapide et économique. Les données préliminaires montrent que 430 de ces produits ont une bonne propriété anticonvulsive. Une évaluation plus complète de 352 de ces produits a permis d'en identifier 16 qui présentent une propriété anticonvulsive à des doses inférieures à 75 mg/kg et qui possèdent un indice de protection supérieur à 5.0.

(J.-L. Gastaut, *Marseilles*)

RESUMEN

El Proyecto de Selección de Anticonvulsivos ha valorado la actividad de 1,495 compuestos que han aparecido en los últimos 2 años mediante el test de electroshock máximo, el test de determinación de umbral epiléptico con la inyección subcutánea de pentilentetrazol y el test de Rotorod de mínima neurotoxicidad. Un protocolo de tres selecciones para valorar estos compuestos ha demostrado ser fiable, rápido y económico. Los resultados preliminares muestran que 430 de estos compuestos tienen buena actividad anticonvulsiva. Evaluaciones completas de 352 productos identificaron 16 que poseen actividad antiepiléptica a dosis menores de 75 mg/kg e índice protectores mayores de 5.0.

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ZUSAMMENFASSUNG

Mit Hilfe des maximalen Elektroshock-Tests, des subkutanen Pentylentetrazol-Krampfschwellentests und des Rotorod minimalen Neurotoxizitäts-Test wurden im Antikonvulsiva-Screening-Projekt in den ersten 2 Jahren die Aktivität von 1;495 eingereichten Substanzen experimentell untersucht. Ein Drei-Raster-Protokoll zur Einschätzung dieser Substanzen wurde als durchführbar, schnell und billig befunden. Die vorläufigen Ergebnisse zeigen, daß 430 dieser Substanzen gute antikonvulsive Wirkung haben. Die endgültige Beurteilung ergab, daß 16 von 352 Substanzen eine antikonvulsive Wirkung bei Dosen unter 75 mg/kg und einen Schutzindex über 5.0 besitzen.

(D. Scheffner, *Heidelberg*)